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EXAMINER	
SWOPE, SHERIDAN	

ART UNIT	PAPER NUMBER
1652	

NOTIFICATION DATE	DELIVERY MODE
10/01/2010	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No.	Applicant(s)	
	10/573,821	OKUNO ET AL.	
	Examiner	Art Unit	
	SHERIDAN SWOPE	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *16 June 2010*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *36-68* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *36-68* is/are rejected.

7)☐ Claim(s) *59 and 60* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *0810*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicants' filing of June 16, 2010, in response to the action mailed February 16, 2010, is acknowledged. It is acknowledged that all previously pending claims have been cancelled and new Claims 36-68 have been added. Claims 36-68 are pending. The invention under prosecution is directed to a method for cleavage using E. coli OmpT protease or a variant thereof having a substitution of Asp^{97} with Ala, Leu, Phe, Met, Ser, Thr, Cys, Asn, Gln, Glu, or His. Claims 36-68 are herein considered.

Priority

For the instant claims, priority is granted to PCT/JP04/14704, filed September 29, 2004, which disclosed the elected invention. The examiner cannot consider whether JP 2003-342183, filed September 20, 2003, disclosed the recited invention because an English translation thereof has not been made available by applicants.

Claim Objections

For Claim 59, the phrase "P10 and P3 positions" should be corrected to "the P10 and P3 positions".

For Claim 60, the phrase "P5 and P3 positions" should be corrected to "the P5 and P3 positions".

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Claims 36-68 are rejected under 35 U.S.C. 101 because the claimed recitation of a use, in this case E. coli OmpT proteases and variants thereof, without setting forth any steps involved in

the process, results in an improper definition of a process, i.e., results in a claim which is not a proper process claim under 35 U.S.C. 101. See for example *Ex parte Dunki*, 153 USPQ 678 (Bd.App. 1967) and *Clinical Products, Ltd.* v. *Brenner*, 255 F. Supp. 131, 149 USPQ 475 (D.D.C. 1966).

Claim Rejections - 35 USC § 112-Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

The prior rejection of claims, because the phrase "E. coli OmpT protease" is indefinite, is withdrawn because all prior claims have been cancelled.

Regarding the current use of the phrase "E. coli OmpT protease" Applicants make the following arguments. The Office's comments are provided in each reply.

(A) The Office's rejection is based, in part on the results of a BLAST sequence alignment with GenBank Acc. No. AAA24430.1, specifically referring to an alignment of Acc. No. AAA24430.1 with Acc. No. YP_444072.1. The GenBank description provided in Acc. No. YP_444072.1, attached as Exhibit 2, discloses that the protein is encoded by an "OmpT" gene. The skilled artisan clearly understood that the protein of Acc. No. YP_444072.1 was (1) from E. coli and (2) an OmpT protease, as seen by the annotation of the sequence in Acc. No. YP_444072.1. The skilled artisan clearly understands which proteins are encompassed by the term "E. coli OmpT protease." Nothing more is needed to comply with 35 U.S.C. § 112, second paragraph. See Moore, 169 USPQ at 238.

(A) Reply: It is acknowledged that GenBank annotates the protein of YP_444072.1 as "outer membrane protease [Escherichia coli]". However, the record in GenBank, and references cited therein, fails to provide any evidence that the protein of YP_444072.1 has the enzymatic activity of an OmpT protease or, in fact, any protease activity. GenBank's annotation that the protein of YP_444072.1 is an outer membrane protease is based on homology with SwissProt/ UniProt P58603. However, annotation by SwissProt/UniProt that P58603 has OmpT protease activity is also based on homology, rather than experimental evidence. As is known in the art, annotation of the "function" for a protein based solely on homology has led to an iterative "transitive identification catastrophe", wherein protein A has the specific function but serial homology analysis does not provide confidence that protein Z has the same function (Barker et al, 2001; pg 30, parg 1). Likewise, the authors of Pfam assert only that their analysis of known sequences is a means to divide proteins into structurally similar categories; no assertion of functional relationships has been made (Sonnhammer et al, 1998; Bateman et al, 2004; Finn et al, 2006). In addition, as explained in the prior action, the functional limitations of the E. coli OmpT of Sugimura et al, 1988a, i.e., "OmpT protease activity" are also not clearly defined (see the Action of February 16, 2010, pgs 4-6).

(B) The Office's remaining allegations concern uncertainties in the art about OmpT protease substrate specificities. Even if true, for the sake of argument, the allegations have no relevance to

whether the skilled artisan would recognize what is meant by an "E. coli OmpT protease." Chemical entities encompassed by a claim do not have to fall within an arbitrary degree of structural and/or functional similarity to comply with 35 U.S.C. § 112, second paragraph. See Moore, 169 USPQ at 238; cf. In re Borkowski, 164 USPQ 642 (CCPA 1970). Nor is there a statutory requirement that the artisan needs to know the precise substrate specificities of E. coli OmpT proteases. For compliance with 35 U.S.C. § 112, second paragraph, the artisan just has to be able to determine the boundaries of the claim. The artisan can, as seen from the evidence on the record. The rejection thus is unsubstantiated and must be withdrawn.

(B) Reply: It is acknowledged that chemical entities encompassed by a claim do not have to fall within an arbitrary degree of structural and/or functional similarity to comply with 35 U.S.C. § 112, second paragraph. The instant rejection is not based on such an arbitrary requirement. Nonetheless, as acknowledged by Applicants, the artisan has to be able to determine the boundaries of the claims. Neither the specification nor the prior art describe the structure or function of E. coli OmpT proteases such that the skilled artisan is able to determine the boundaries of the claims.

Based on Applicants' arguments, for purposes of examination, it is assumed that "E. coli OmpT protease" means any E. coli protein having any structure and having any activity of the E. coli OmpT protease of Sugimura et al, 1988a (AAA24430.1) and that the recited OmpT Asp^{97} variants encompass any variant of said E. coli proteins having any structure comprising a substitution of a residue corresponding to Asp^{97} of the E. coli OmpT protease of Sugimura et al, 1988a (AAA24430.1).

Claims 36-68 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention for the following reasons.

Claims 36-68 provide for the use of E. coli OmpT protease and variants thereof; but, since the claim does not set forth any steps involved in the method/process, it is unclear what method/process applicant is intending to encompass. A claim is indefinite where it merely recites a use without any active, positive steps delimiting how this use is actually practiced.

Claims 36, 51, 55, 59, and 60, and claims dependent therefrom, are rendered indefinite for improper antecedent usage as follows.

For Claim 36, penultimate line, "the sequence" lacks antecedent basis.

For Claim 36, the phrases "a P10 position" and "a P3 position" should be corrected to "the P10 position" and "the P3 position".

For Claim 51 "the amino acid sequence" lacks antecedent basis.

For Claim 51, the phrases "a P10 position" and "a P3 position" should be corrected to "the P10 position" and "the P3 position".

For Claim 55, the phrase "The method" should be corrected to "The process".

For Claim 55 "the amino acid sequence" lacks antecedent basis.

For Claim 55, the phrases "a P10 position" and "a P3 position" should be corrected to "the P10 position" and "the P3 position".

Regarding Claim 67, it is assumed that the phrase "the target peptide is composed of between 22 and 45 amino acid residues" means "the target peptide consists of between 22 and 45 amino acid residues".

Any subsequent rejection based, on clarification of the above phrases and terms, will not be considered a new ground for rejection.

Claim Rejections - 35 USC § 112-First Paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Enablement

These rejections are based on the assumption that that "E. coli OmpT protease" means any E. coli protein having any structure and having any activity of the E. coli OmpT protease of

Sugimura et al, 1988a (AAA24430.1) and that the recited OmpT Asp^{97} variants encompass any variant of said E. coli proteins having any structure comprising a substitution of a residue corresponding to Asp^{97} of the E. coli OmpT protease of Sugimura et al, 1988a (AAA24430.1). These rejections are also based on the assumption that a desired cleavage motif may consist of up to 15 amino acid residues (original Claim 1), which represents 3.3×10^{19} sequences. With an Arg or Lys at P1, the genus of cleavage motifs is 3.3×10^{18} sequences.

E. coli proteins having an activity of AAA24430.1

Claims 36-47 and 66 are rejected under 35 U.S.C. 112, first paragraph/enablement for the following reasons. Claims 36-47 and 66 are directed to a method using any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, to cleave any motif comprising an P1 Arg or Lys, a P1' other than Asp, Glu, or Pro, and two or three consecutive basic residues within P10-P3.

The specification is enabling for cleaving three motifs by the E. coli OmpT protease of Sugimura et al, 1988a (AAA24430.1), as disclosed by Fig. 3, 5, and 11. The art is enabling for cleaving certain motifs by the E. coli OmpT protease of Sugimura et al, 1988a (AAA24430.1), as described by the rejections under 35 USC 102 and 103(a), below. However, the specification does not reasonably provide enablement for using any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, to cleave any motif comprising an P1 Arg or Lys, a P1' other than Asp, Glu, or Pro, and two or three consecutive basic residues within P10-P3. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

In regards to this enablement rejection, the application disclosure and claims are compared per the factors indicated in the decision In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988). These factors are considered when determining whether there is sufficient evidence to support a description that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is undue. The factors include but are not limited to: (1) the nature of the invention; (2) the breath of the claims; (3) the predictability or unpredictability of the art; (4) the amount of direction or guidance presented; (5) the presence or absence of working examples; (6) the quantity of experimentation necessary; (7) the relative skill of those skilled in the art. Each factor is here addressed on the basis of a comparison of the disclosure, the claims, and the state of the prior art in the assessment of undue experimentation.

Claims 36 and 42-45 are so broad as to encompass any method for cleaving any motif comprising an P1 Arg or Lys, a P1' other than Asp, Glu, or Pro, and two or three consecutive basic residues within P10-P3 or P5-P3, wherein the method uses any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1.

Claims 37, 38, 40, and 41 are so broad as to encompass any method for cleaving any motif in a fusion protein, wherein cleavage is between a protecting peptide and a target peptide of the fusion protein, wherein the motif comprises an P1 Arg or Lys, a P1' other than Asp, Glu, or Pro, and two or three consecutive basic residues within P10-P3, wherein P1 is the C-terminal residue of the protecting peptide and P1' is the N-terminal residue of the target protein, wherein the method uses any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1.

Claim 39 is so broad as to encompass any method for cleaving any motif in a fusion protein, wherein cleavage is between a protecting peptide and a target protein of either ACTH (1-24), motilin, or calcitonin precursor of the fusion protein, wherein the motif comprises an P1 Arg or Lys, a P1' other than Asp, Glu, or Pro, and two or three consecutive basic residues within P10-P3, wherein P1 is the C-terminal residue of the protecting peptide and P1' is the N-terminal residue of the target protein, wherein the method uses any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1.

Claims 42 and 66 are so broad as to encompass inhibiting cleavage at any motif cleaved by any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein said inhibiting is accomplished by setting the P3 position in the motif to an acidic amino acid.

Claims 46 and 47 are so broad as to encompass any method for cleaving any motif comprising SEQ ID NO: 11 or 12, wherein the method uses any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1.

The scope of these claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of methods broadly encompassed by the claims. Since the amino acid sequence of a protease determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity to cleave the recited genera of motifs requires a knowledge of and guidance with regard to which amino acid substitutions allow cleavage of which motifs and detailed knowledge of the ways in which the protease's structure relates to its function to cleave any specific motif. However, in this case the disclosure is limited to the three specific

combinations of E. coli protease AAA24430.1 and motifs disclosed by Fig. 3, 5, and 11 and anticipated or rendered obvious by the prior art, as described by the rejections under 35 USC 102 and 103(a), below.

While methods for isolating variant proteases and testing said variants for cleavage of specific motifs are known, it is not routine in the art to use any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, for cleaving any of the ~3.3 x 10^{18} motifs encompassed by Claims 36-47 and 66. Neither the art nor the specification provides sufficient guidance such that the required experimentation would not be undue. Neither the specification nor the prior art defined the functional or structural metes and bounds of the encompassed genus of any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1. Moreover, neither the specification nor the prior art provide sufficient guidance, such that determining which of the extremely large number of encompassed motifs would be cleaved by any said E. coli protease, would not be undue.

The specification does not support the broad scope of Claims 36-45, which encompasses all methods for cleaving any motif comprising a P1 Arg or Lys, a P1' other than Asp, Glu, or Pro, and two or three consecutive basic residues within P10-P3 or P5-P, including fusion proteins wherein P1 is the C-terminal residue of the protecting peptide and P1' is the N-terminal residue of the target protein. The specification does not support the broad scope of Claims 42 and 66, which encompasses all methods for inhibiting cleavage at any motif cleaved by any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein said inhibiting is accomplished by setting the P3 position in the motif to

an acidic amino acid. The specification does not support the broad scope of Claims 46 and 47, which encompasses all methods for cleaving any motif comprising SEQ ID NO: 11 or 12, wherein the method uses any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1. The specification does not support the broad scope of Claims 36-47 and 66 because the specification does not establish: (A) any E. coli protein, other than the OmpT protease set forth by AAA24430.1, having the desired activity; (B) which E. coli proteins, having any activity of the OmpT protease set forth by AAA24430.1, cleave which encompassed motifs; (C) all amino acid residues in positions P10-P2 and P2'-P5', and specific combinations thereof, that support cleavage by any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1; (D) amino acid residues in positions P10-P2 and P2'-P5', and specific combinations thereof, that inhibit cleavage by any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1; (E) residues of the desired cleavage motif which may, or may not, be modified without affecting the desired cleavage activity; (F) the general tolerance of the cleavage activity to modification of any cleavage motif and extent of such tolerance; (G) a rational and predictable scheme for modifying any residues in a cleavage motif with an expectation of obtaining the desired cleavage activity by any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1; (H) the structure of all peptides having calcitonin precursor activity (Claim 39); and (I) the specification provides insufficient guidance as to which of the essentially infinite possible choices of combinations of E. coli proteins and cleavage motifs is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including any method for cleaving essentially any motif with any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of the identity of methods having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Asp^{97} variants of E. coli proteins having an activity of AAA24430.1

Claims 48-65, 67, and 68 are rejected under 35 U.S.C. 112, first paragraph/enablement for the following reasons. Claims 48-65, 67, and 68 are directed to a method using any variant of any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising a substitution at the residue corresponding to at Asp^{97} of AAA24430.1, wherein the method uses any such variant to cleave any motif comprising (i) an P1 Arg or Lys, a P1' other than Asp, Glu, or Pro, and two or three consecutive basic residues within P10-P3 (Claim 48), (ii) an P1 Arg or Lys and a P1' other than Arg or Lys (Claim 49), or (iii) an P1 Arg or Lys, a P1' other than Arg or Lys, and a single basic residue or two or three consecutive basic residues within P10-P3 (Claim 51).

The specification is enabling for using variants of the E. coli OmpT protease taught by Sugimura et al, 1988a (AAA24430.1), wherein the variant has a substitution at Asp^{97}, as follows:

- Asp^{97}Met OmpT to cleave the motifs AAR↓RR↓AR↓FVPIF, ADR↓RR↓AR↓FVPIF, and DARRR↓AR↓FVPIF (Fig5,15), and to minimally cleave LRLYR↓[A/V/F/S/C/Y]HHGS (Exmp13;Table 1)
- Asp^{97}Leu OmpT to cleave the motif RAR↓SYSME (Fig11-12), and to minimally cleave the motifs LRLYR↓[A/F/S/C/Y]HHGS (Exmp13&Table 1)
- Asp^{97}His OmpT to cleave the motifs AAR↓RR↓AR↓CGNLS (Fig11-12) and to minimally cleave the motifs LRLYR↓[A/V/I/F/M/S/T/C/N/K/R]HHGS (Exmp13;Table 1)
- Asp^{97}Ala OmpT to minimally cleave the motif LRLYR↓[A]HHGS (Exmp13;Table 1)
- Asp^{97}Phe OmpT to minimally cleave the motif LRLYR↓[A]HHGS (Exmp13;Table 1)
- Asp^{97}Ser OmpT to minimally cleave the motifs LRLYR↓[A/C]HHGS (Exmp13;Table 1)
- Asp^{97}Thr OmpT to minimally cleave the motifs LRLYR↓[A/V/F/S/C/K/R]HHGS (Exm13;Table 1)
- Asp^{97}Gln OmpT to minimally cleave the motifs LRLYR↓[A/S]HHGS (Exmp13;Table 1)
- Asp^{97}Asn OmpT to minimally cleave the motifs LRLYR↓[A/S/C]HHGS (Exmp13;Table 1)

The prior art is enabling for cleaving certain motifs by Asp^{97} variants of the E. coli OmpT protease of Sugimura et al, 1988a (AAA24430.1), as described in the rejections under 35 USC 103(a) below.

However, the specification does not reasonably provide enablement for using any variant of any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising a substitution at the residue corresponding to Asp^{97} of AAA24430.1, to cleave any motif comprising (i) a P1 Arg or Lys, a P1' other than Asp, Glu, or Pro, and two or three consecutive basic residues within P10-P3 (Claim 48), (ii) a P1 Arg or Lys and a P1' other than Arg or Lys (Claim 49), or (iii) an P1 Arg or Lys, a P1' other than Arg or Lys, and a single basic residue or two or three consecutive basic residues within P10-P3 (Claim 51). The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

In regards to this enablement rejection, the application disclosure and claims are compared per the factors indicated in the decision In re Wands 858 F.2d 731, 8 USPQ2nd 1400

(Fed. Cir, 1988). These factors are considered when determining whether there is sufficient evidence to support a description that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is undue. The factors include but are not limited to: (1) the nature of the invention; (2) the breath of the claims; (3) the predictability or unpredictability of the art; (4) the amount of direction or guidance presented; (5) the presence or absence of working examples; (6) the quantity of experimentation necessary; (7) the relative skill of those skilled in the art. Each factor is here addressed on the basis of a comparison of the disclosure, the claims, and the state of the prior art in the assessment of undue experimentation.

Claim 48 is so broad as to encompass any method for cleaving any motif, comprising a P1 Arg or Lys, a P1' other than Asp, Glu, or Pro, and two or three consecutive basic residues within P10-P3, wherein the method uses any variant of any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising any substitution of A, L, F, M, S, T, C, N, Q, or H at a residue corresponding to D^{97} of AAA24430.1.

Claims 49, 50, and 63-65 are so broad as to encompass any method for cleaving any motif, comprising a P1 Arg or Lys and a P1' other than Arg or Lys, wherein the method uses any variant of any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising any substitution of A, L, F, M, S, T, C, N, Q, or H at a residue corresponding to D^{97} of AAA24430.1.

Claim 51-53, 59, and 60 are so broad as to encompass any method for cleaving any motif, comprising a P1 Arg or Lys, a P1' other than Arg or Lys, and a single basic residue or two or three consecutive basic residues within P10-P3 or P5-P3, wherein the method uses any variant of

any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising any substitution of A, L, F, M, S, T, C, N, Q, or H at a residue corresponding to D^{97} of AAA24430.1.

Claims 54-56 and 67 are so broad as to encompass any method for cleaving any fusion protein at a motif comprising a P1 Arg or Lys and a P1' other than Arg or Lys, wherein P1' is the C-terminal residue of a protecting peptide and P1' is the N-terminal residue of a target protein, wherein the method uses any variant of any E. coli protein having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising any substitution of A, L, F, M, S, T, C, N, Q, or H at a residue corresponding to D^{97} of AAA24430.1.

Claims 57 and 58 are so broad as to encompass inhibiting cleavage at any motif cleaved by any variant of any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising any substitution of A, L, F, M, S, T, C, N, Q, or H at D^{97}, and wherein said inhibiting is accomplished by setting the P3 residue in any said motif to an acidic amino acid.

Claims 61 and 62 are so broad as to encompass any method for cleaving any motif comprising SEQ ID NO: 11 or 12, wherein the method uses any variant of any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising any substitution of A, L, F, M, S, T, C, N, Q, or H at a residue corresponding to D^{97} of AAA24430.1.

Claim 68 is so broad as to encompass any method for cleaving any motif in a fusion protein, wherein cleavage is between a protecting peptide and a target protein of either ACTH (1-

24), motilin, or calcitonin precursor of the fusion protein, wherein the motif comprises a P1 Arg or Lys and a P1' Arg or Lys, wherein the method uses any variant of any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising any substitution of A, L, F, M, S, T, C, N, Q, or H at a residue corresponding to D^{97} of AAA24430.1.

The scope of these claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of methods broadly encompassed by the claim. Since the amino acid sequence of a protease determines its structural and functional properties, predictability of which changes can be tolerated in a protease's amino acid sequence and obtain the desired activity to cleave the recited genera of motifs requires a knowledge of and guidance with regard to which amino acid alterations allow cleavage of which motifs and detailed knowledge of the ways in which the protease's structure relates to its function to cleave any specific motif. However, in this case the disclosure is limited to the specific combinations of protease variants and motifs listed above.

While methods for isolating and making variant proteins and testing said variants for cleavage of specific motifs are known, it is not routine in the art to screen the encompassed large number of protease variants for cleavage at any motif comprising essentially any amino acid at positions P10-P5', as encompassed by the instant claims. Neither the art nor the specification provides sufficient guidance such that the required experimentation would not be undue. Regarding which proteases to be used, neither the specification nor the art defined the substrate specificity such that the skilled artisan would be apprized of the functional or structural metes and bounds of any E. coli protease having any structure and any activity of the E. coli OmpT

protease taught by Sugimura et al, 1988a (AAA24430.1), or any Asp^{97} variants thereof. Regarding motifs to be cleaved, the art teaches that Asp^{97} of AAA24430.1 OmpT protease is critical for P1' cleavage motif recognition and that the $Asp^{97}Ala$ substitution dramatically reduces the efficacy of cleavage at -Arg↓Arg-, i.e., to only 6% (Kramer et al, Fig2-4; pg429, parg1). In contrast, the instant specification teaches that the $Asp^{97}Ala$ AAA24430.1 OmpT protease variant has 70% of the parent activity (Table 1). Thus, cleavage motifs for the AAA24430.1 OmpT protease variants, as well as E. coli proteins having any activity of AAA24430.1, remained unpredictable. Moreover, the difference in the results of Kramer et al and the instant specification strongly argue that the amino acid residues at positions P10-P2 and P2'-P5' affect, in a manner unpredictable at the time of filing, the ability of any peptide motif to be cleaved by the encompassed protease variants.

The claims encompass using variants of E. coli proteases for cleaving a desired motif comprising up to 15 amino acid residues (positions P10-P5'). It is acknowledged that a P1 Arg is highly favored for cleavage by the E. coli OmpT protease set forth by AAA24430.1 (Kramer et al; Fig 4). It is also acknowledged that, based on the art and the specification, the skilled artisan would, more likely than not, be able to predict the favored amino acid(s) at the P1' position (in combination with a P1 Arg) for the encompassed AAA24430.1 E. coli protease variants (see rejections under 35 USC 103(a)). However, neither the specification nor the art defined the substrate specificity, encompassing up to positions P10-P5', such that the skilled artisan would be apprized of the functional metes and bounds of the E. coli OmpT protease (AAA24430.1) per se. Moreover, little information is provided regarding these positions and cleavage by the encompassed AAA24430.1 protease variants or variants of any E. coli protein

having any activity of AAA24430.1; the specification and the prior art provide little evidence as to which amino acid residues are favored, permitted, or non-favored at positions P10-P2 and P2'-P5', for cleavage by the large genus of encompassed variants. Even assuming that the skilled artisan would be able to predict which amino acids are favored at the P1 and P1' positions, the genus of all motifs encompassed by residues P10-P2 and P2'-P5' is represented by 20^{13} i.e., 8.2 x 10^{16} sequences, which would need to be tested with the encompassed large number of protease variants. This represents much greater than 8.2 x 10^{17} assays. While enablement is not precluded by the necessity for routine screening, if a large amount of screening is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. Sufficient guidance has not been provided in the instant specification.

The specification does not support the broad scope of Claims 48-65, 67, and 68, which encompasses the methods listed above. The specification does not support the broad scope of Claims 48-65, 67 and 68 because the specification does not establish: (A) which E. coli protein, other than the OmpT protease set forth by AAA24430.1, have any activity of AAA24430.1; (B) any variant of any E. coli protein, other than a few variants of AAA24430.1, having the desired activities; (C) amino acid residues in positions P10-P2 and P2'-P5', and specific combinations thereof, that support cleavage by any Asp^{97} E. coli protein variant; (D) amino acid residues in positions P10-P2 and P2'-P5', and specific combinations thereof, that inhibit cleavage by any specific Asp^{97} OmpT protease variant; (E) residues of the desired cleavage motif which may, or may not, be modified without affecting the cleavage activity; (F) the general tolerance of the cleavage activity to modification of any desired cleavage motif and extent of such tolerance; (G)

a rational and predictable scheme for modifying any residues in a cleavage motif, in conjunction with modification of the Asp^{97} residue of any E. coli protein, with an expectation of obtaining the desired cleavage activity; (H) the structure of all peptides having calcitonin precursor activity (Claim 68); and (I) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including any method for cleaving essentially any motif with any variant of any E. coli protein having any activity of the E. coli OmpT protease of AAA24430.1, wherein the variant has any substitution of Ala, Leu, Phe, Met, Ser, Thr, Cys, Asn, Gln, or His at a position corresponding to Asp^{97} of AAA24430.1. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of the identity of methods having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

In support of their request that the prior, analogous rejection be withdrawn, Applicants provide the following arguments. These arguments are not found to be persuasive for the reasons following each argument.

(A) The Office must provide objective evidence or reasoning that the skilled artisan would doubt the enablement provided by the specification. See In re Cortright, 165 F.3d 1353, 1357, 49 USPQ2d 1464 (Fed. Cir. 1999). The Office, however, only questions whether all motifs would be cleaved with equal efficiency, not whether OmpT could not cleave the motifs. It is possible that OmpT does not cleave some motifs encompassed by the claims at all. Even in an unpredictable art, however, long established judicial precedent holds that exemplification of each and every embodiment encompassed by a claim is not required to comply with 35 U.S.C. § 112, first paragraph. See In re Angstadt, 190 USPQ 214, 218 (CCPA 1976). Applicants provide the degree of exemplification required by law, so the rejection should be withdrawn.

(A) Reply: It is acknowledged that exemplification of each and every embodiment encompassed by a claim is not required to comply with 35 U.S.C. § 112, first paragraph. However, the specification must enable the skilled artisan to make and use the full scope of the recited invention. Determining which embodiments have the desired structural and functional characteristics must not require undue experimentation.

(B) The Office alleges that the disclosure of Kramer provides objective evidence of non-enablement. Referring to Kramer, p. 429, ¶1, and FIGS. 2-4, the Office alleges that Asp97 is "critical" for cleavage motif recognition and that altering Asp97 changes cleavage efficacy. See Office Action, p. 13, ¶2. To the contrary, Kramer states (p. 429, lst col., ¶1): D97A OmpT displayed only 6% residual activity; therefore, we propose that Asp97 is responsible for the observed P1' specificity.

Kramer uses the following substrate (p. 427, 1st col., ¶3):

Abz -Ala-Arg-Arg-Ala- Dap (dnp)-Gly

P2 –P1↓P1'-P2',

where Abz is o-aminobenzoyl, and Dap(dnp) is N-13-dinitrophenyl-L-diaminopropionic acid. FIG. 4 of Kramer shows that this substrate is cleaved between the two arginine residues. See also Kramer et al., Eur. J. Biochem. 267:885-893 (2000), p. 887, 2nd col., 4th line from the bottom.

(B) Reply: It is acknowledged that Kramer et al teaches that the D97A OmpT variant has only 6% residual activity for Arg-Arg and concludes that position 97 of OmpT protease is responsible for the P1' specificity of the cleavage motif for the parent and variant proteases.

Importantly, as explained in the prior action, the results of Kramer et al, showing only 6% activity for cleavage between Arg↓Arg disagrees with Applicants' results showing that the $D^{97}A$ OmpT variant had 70% of the activity of the parent protease for Arg↓Arg (Table 1). Thus, as also explained in the prior action, cleavage by the recited OmpT protease variants remained unpredictable and the specification provides little evidence as to which amino acid residues are favored, permitted, or non-favored at positions P10-P2 and P2-P5', for cleavage by the D97A variant and the other recited variants.

It is acknowledged that a considerable amount of experimentation is permissible if it is merely routine or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. In the instant case, the lack of guidance regarding the structure and cleavage specificity for the encompassed variants together with the scope of the 3.3×10^{18} encompassed sequences renders determining which combinations of proteins and substrates have the desired characteristics undue.

For these reasons and those explained in the prior action, Claims 48-65, 67, and 68 are rejected under 35 U.S.C. 112, first paragraph/enablement.

Written Description

These rejections are based on the assumption that that (i) "E. coli OmpT protease" means any E. coli protein having any structure and having any activity of the E. coli OmpT protease of Sugimura et al, 1988a (AAA24430.1), (ii) that the recited OmpT Asp^{97} variants are derived from said any E. coli protein, and (iii) a desired cleavage motif may consist of up to 15 amino acid

residues (original Claim 1), which represents 3.3 x 10^{19} sequences. With an Arg or Lys at P1, the genus of cleavage motifs is 3.3 x 10^{18} sequences.

E. coli proteins having an activity of AAA24430.1

Claims 36-47 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Claims 36-47 are directed to a genus of methods for cleaving any motif comprising an P1 Arg or Lys, a P1' other than Asp, Glu, or Pro, and two or three consecutive basic residues within P10-P3 or P5-P3, including (i) any motif comprising SEQ ID NO: 11 or 12 and (ii) fusion proteins wherein P1 is the C-terminal residue of the protecting peptide and P1' is the N-terminal residue of the target protein, wherein the method uses any E. coli protein having any activity of the E. coli OmpT proteinase of AAA24430.1. The specification teaches only a three representative species of such methods (Fig 3, 5, and 11). Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a method for cleaving any motif comprising an P1 Arg or Lys, a P1' other than Asp, Glu, or Pro, and two or three consecutive basic residues within P10-P3 or P5-P, wherein the method uses any E. coli protein having any activity of the E. coli OmpT proteinase of AAA24430.1. Given this lack of description of representative species encompassed by the genera of the claims, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claims 42 and 66 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Claims 42 and 66 are directed to a genus of methods for inhibiting cleavage at any motif cleaved by any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein said inhibiting is accomplished by setting the P3 position in the motif to an acidic amino acid. The specification teaches only a single representative species of such methods (Fig 4). Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a method for inhibiting cleavage at any motif cleaved by any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein said inhibiting is accomplished by setting the P3 position in the motif to an acidic amino acid. Given this lack of description of representative species encompassed by the genera of the claims, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Asp^{97} variants of E. coli proteins having an activity of AAA24430.1

Claim 48 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. This claim is directed to a genus of methods for cleaving essentially any motif having a P1 Arg or Lys, a P1' other than Asp, Glu, or Pro, and two or three consecutive

basic residues within P10 to P3 using any variant of any E. coli protein having any substitution of A, L, F, M, S, T, C, N, Q, or H at a position corresponding to residue D^{97} of OmpT protease AAA24430.1. Out of the 3.3×10^{18} encompassed motifs, the specification teaches only three species of said method, wherein the $D^{97}M$ cleaves RRRAR↓F-motilin (Fig 5), $D^{97}L$ cleaves RRRAR↓S-ACTH (Fig 12), or $D^{97}H$ cleaves RRRAR↓C-calcitonin (Fig 12), wherein all variants are variants of AAA24430.1. Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a method for cleaving a essentially any motif having a P1 Arg or Lys, a P1' other than Asp, Glu, or Pro, and two or three consecutive basic residues within P10 to P3 using any variant of any E. coli protein having any substitution of A, L, F, M, S, T, C, N, Q, or H at a position corresponding to residue D^{97} of OmpT protease AAA24430.1. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claims 49, 50, and 63-65 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Claims 49, 50, and 63-65 are directed to a genus of methods for cleaving any motif, comprising a P1 Arg or Lys and a P1' other than Arg or Lys, wherein the method uses any variant of any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising any substitution of A, L, F, M, S, T, C, N, Q, or H at a residue corresponding to D^{97}

of AAA24430.1. Out of the 3.3×10^{18} encompassed motifs encompassed, the specification teaches the structure of only 14 that can be cleaved by the $D^{97}M$ variant, 6 that can be cleaved by the $D^{97}L$ variant, 12 that can be cleaved by the $D^{97}H$ variant, one that can be cleaved by the $D^{97}A$ variant, one that can be cleaved by the $D^{97}F$ variant, two that can be cleaved by the $D^{97}S$ variant, 6 that can be cleaved by the $D^{97}T$ variant, two that can be cleaved by the $D^{97}Q$ variant, and three that can be cleaved by the $D^{97}N$ variant, wherein all said protease variants are variants of AAA24430.1. Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a method for cleaving any motif, comprising a P1 Arg or Lys and a P1' other than Arg or Lys, wherein the method uses any variant of any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising any substitution of A, L, F, M, S, T, C, N, Q, or H at a residue corresponding to D^{97} of AAA24430.1. Given this lack of description of representative species encompassed by the genera of the claims, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claim 51-53, 59, and 60 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Claim 51-53, 59, and 60 are directed to a genus of methods for cleaving any motif, comprising a P1 Arg or Lys, a P1' other than Arg or Lys, and a single basic residue or two or three consecutive basic residues within P10-P3 or P5-P3, wherein

the method uses any variant of any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising any substitution of A, L, F, M, S, T, C, N, Q, or H at a residue corresponding to D^{97} of AAA24430.1. Out of the 3.3×10^{18} encompassed motifs comprising a single basic residue within P10-P3 or P5-P3, the specification teaches only the methods of cleavage outlined in the rejection under USC 35, 112/enablement, above. Out of the 3.3×10^{18} encompassed motifs comprising two or three consecutive basic residues within P10-P3 or P5-P3, the specification teaches only three species of said method, wherein the $D^{97}M$ cleaves RRRAR↓F-motilin (Fig 5), $D^{97}L$ cleaves RRRAR↓S-ACTH (Fig 12), or $D^{97}H$ cleaves RRRAR↓C-calcitonin (Fig 12), wherein all variants are variants of AAA24430.1. Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a method for cleaving any motif, comprising a P1 Arg or Lys, a P1' Arg or Lys, and a single basic residue or two or three consecutive basic residues within P10-P3 or P5-P3, wherein the method uses any variant of any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising any substitution of A, L, F, M, S, T, C, N, Q, or H at a residue corresponding to D^{97} of AAA24430.1. Given this lack of description of representative species encompassed by the genera of the claims, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claims 54-56 and 67 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably

convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Claims 54-56 and 67 are directed to a genus of methods for cleaving any fusion protein at a motif comprising a P1 Arg or Lys and a P1' other than Arg or Lys, wherein P1 is the C-terminal residue of a protecting peptide and P1' is the N-terminal residue of a target protein, wherein the method uses any variant of any E. coli protein having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising any substitution of A, L, F, M, S, T, C, N, Q, or H at a residue corresponding to D^{97} of AAA24430.1. The specification teaches only three representative species of such methods (Fig 6 and 12). Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a method for cleaving any fusion protein at a motif comprising a P1 Arg or Lys and a P1' other than Arg or Lys, wherein P1 is the C-terminal residue of a protecting peptide and P1' is the N-terminal residue of a target protein, wherein the method uses any variant of any E. coli protein having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising any substitution of A, L, F, M, S, T, C, N, Q, or H at a residue corresponding to D^{97} of AAA24430.1. Given this lack of description of representative species encompassed by the genera of the claims, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claims 57 and 58 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one

skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Claims 57 and 58 are directed to a genus of methods for inhibiting cleavage at any motif cleaved by any variant of any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising any substitution of A, L, F, M, S, T, C, N, Q, or H at D^{97}, and wherein said inhibiting is accomplished by setting the P3 residue in any said motif to an acidic amino acid. The specification teaches no representative species of such methods. Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a method for inhibiting cleavage at any motif cleaved by any variant of any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising any substitution of A, L, F, M, S, T, C, N, Q, or H at D^{97} and any function, and wherein said inhibiting is accomplished by setting the P3 residue in any said motif to an acidic amino acid. Given this lack of description of representative species encompassed by the genera of the claims, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claims 61 and 62 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Claims 61 and 62 are directed to a genus of methods for cleaving any motif comprising SEQ ID NO: 11 or 12, wherein the method uses any variant of

any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising any substitution of A, L, F, M, S, T, C, N, Q, or H at a residue corresponding to D^{97} of AAA24430.1. The specification teaches only three representative species of such methods (Fig 5&11). Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a method for cleaving any motif comprising SEQ ID NO: 11 or 12, wherein the method uses any variant of any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising any substitution of A, L, F, M, S, T, C, N, Q, or H at a residue corresponding to D^{97} of AAA24430.1. Given this lack of description of representative species encompassed by the genera of the claims, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claim 68 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Claim 68 is directed to a genus of methods for cleaving any motif in a fusion protein, wherein cleavage is between a protecting peptide and a target protein of either ACTH (1-24), motilin, or calcitonin precursor of the fusion protein, wherein the motif comprises an P1 Arg or Lys derived from the protecting protein and a P1' of other than Asp, Glu, or Pro derived from the target protein, wherein the method uses any variant of any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the

variant has any structure comprising any substitution of A, L, F, M, S, T, C, N, Q, or H at a residue corresponding to D^{97} of AAA24430.1. The specification teaches only three representative species of such methods using the $D^{97}M$, $D^{97}L$, or $D^{97}H$ variant of AAA24430.1 (Fig 5&11). Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a method for cleaving any motif in a fusion protein, wherein cleavage is between a protecting peptide and a target protein of either ACTH (1-24), motilin, or calcitonin precursor of the fusion protein, wherein the motif comprises an P1 Arg or Lys and a P1' other than Asp, Glu, or Pro, wherein the method uses any variant of any E. coli protein, having any structure and any function of the E. coli OmpT protease set forth by AAA24430.1, wherein the variant has any structure comprising any substitution of A, L, F, M, S, T, C, N, Q, or H at a residue corresponding to D^{97} of AAA24430.1. Given this lack of description of representative species encompassed by the genera of the claims, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

In support of their request that the prior, analogous rejections be withdrawn, Applicants provide the following arguments. These arguments are not found to be persuasive for the reasons following each argument.

(A) A fully described genus must allow one skilled in the art to "visualize or recognize the identity of the members of the genus" and to "'distinguish the claimed genus from others." University of California v. Eli Lilly & Co., 119 F.3d 1559, 1568 (Fed. Cir. 1997). "[W]hat is needed to support generic claims to biological subject matter depends on a variety of factors, such as the existing knowledge in the particular field, the extent and content of the prior art, the maturity of the science or technology, [and] the predictability of the aspect at issue." Capon v. Eshhar, 418 F.3d 1349, 1359 (Fed. Cir. 2005). "'[I]t is not necessary that every permutation within a generally operable invention be effective in order for an inventor to obtain a generic claim, provided that the effect is sufficiently demonstrated to characterize a generic invention. See In re Angstadt, 537 F.2d 498, 504 (CCPA 1976); Capon, 418 F.3d at 1359.

(A) Reply: It is acknowledged that that every permutation of an invention need not be described. However, in the instant case, out of 3.6×10^{15} - 3.3×10^{18} encompassed methods for each

E. coli protein variant, the specification has described only a few, or no, functional permutations. Thus, the methods are not described such that the skilled artisan would recognize possession.

For these reasons and those explained in the prior action, Claims 48-60, 63-65 and 67 are rejected under 35 U.S.C. 112, first paragraph/written description.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claim 36 and 44 are rejected under 35 U.S.C. 102(b) as being anticipated by Sugimura et al, 1988a, as evidenced by Okuno et al, 2002b or Dekker et al, 2001. Sugimura et al teaches incubation of the C-terminus of human γ-interferon, Gly-Lys-Arg-Lys-Arg-Ser-Gln-Met-Leu-Phe-Arg-Gly-Arg-Arg-Ala-Ser-Gln, with OmpT protease. Based on the teachings of Okuno et al (Fig2) and Dekker et al (Table 2), which both show that OmpT protease cleaves between Arg↓Ser, the skilled artisan would have known that, more likely than not, said sequence would be cleaved between Gly-Lys-Arg-Lys-Arg↓Ser-Gln-Met-Leu-Phe-Arg-Gly-Arg-Arg-Ala-Ser-Gln. Said sequence comprises two basic residues within P10 and P3. Therefore, Claims 36 and 44 are rejected under 35 U.S.C. 102(b) as being anticipated by Sugimura et al, 1988a as evidenced by Okuno et al, 2002b or Dekker et al, 2001.

Applicants argument regarding the prior rejection based on Sugimura et al, 1988a are not relevant to the rejection above.

Claims 36 and 43-45 are rejected under 35 U.S.C. 102(b) as being anticipated by Stumpe et al, 1998. Stumpe et al teaches that the OmpT protease AAA24430.1 degrades salmon protamine having the sequence MPRRRRSSSRPVRRRRRPRVSRRRRRRGGRRRR (Fig2&4).

Stumpe et al teaches that it was known in the art that OmpT protease cleaves between basic residues (pg 4006, ¶3). Thus, Stumpe et al teaches cleavage of salmon protamine at MPRRRRSSSRPVRRRR↓RPRVS-RRRR↓R↓RGGR↓RRR, wherein said cleavage motifs have two or three consecutive basic residues within P10-P3. Therefore, Claims 36 and 43-45 are rejected under 35 U.S.C. 102(b) as being anticipated by Stumpe et al, 1998.

> In support of their request that the prior, analogous rejection of Claims 4, 5 and 10 under 35 U.S.C. § 103(a) as unpatentable over Stumpe in view of Suzuki and Sugimura (1988a) be withdrawn, Applicants provide the following argument, which is relevant to the rejection above. In the claimed process, two or three consecutive basic residues are situated from a P10 position to a P3 position of the cleaved polypeptide. In Stumpe, however, the major disclosed protamine substrates do not have a sequence that would read on this claim element, regardless of where the peptide was cleaved. Any sequence encompassing eight (Stump) basic residues (P10 - P3) would include more than the recited two or three consecutive basic amino acids.
>
> This argument is not found to be persuasive for the following reason. It is acknowledged that the protamine of Stump has more than three basic residues within P10-P3 of some cleavage motifs. Nonetheless, the protamine of Stump is encompassed by Claims 36 and 43-46 because said claims do not recite a limitation for the upper number of basic residues within P10-P3.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

E. coli proteins having an activity of AAA24430.1

Claims 36 and 43-46 are rejected under 35 U.S.C. 103(a) as being unpatentable over Stumpe et al, 1998 in view of Suzuki et al, 1972 and Kramer et al, 2001. Stumpe et al teaches that protamines are cleaved by OmpT protease AAA24430.1 (Fig2). Stumpe et al does not specifically teach cleavage of a protamine comprising the peptide of SEQ ID NO: 11. Suzuki et al teaches that the protamine component Clupein YII comprises the sequence ^{23}Arg-Arg-Arg-

Ala-Arg-Arg[28] (Fig7), wherein residues 23-27 represent SEQ ID NO: 11. It would have been obvious to a person of ordinary skill in the art to use OmpT protease AAA24430.1 to cleave the specific protamine component Clupein YII between ^{27}Arg↓Arg^{28}, wherein positions P5-P1 are as set forth by SEQ ID NO: 11. Motivation to do so is provide by the desire to demonstrate that OmpT protease AAA24430.1 cleaves the specific protamine component Clupein YII. The expectation of success is high, as Stumpe et al teaches that it "was not unexpected that OmpT is the extracytoplasmic protease that inactivates the highly cationic peptide protamine (pg4006, parg3) and it was known in the art that E. coli OmpT protease AAA24430.1 can cleave between the dibasic residues –Ala-Arg↓Arg-Ala (Kramer et al; Fig4). Therefore, Claims 36 and 43-46 are rejected under 35 U.S.C. 103(a) as being unpatentable over Stumpe et al, 1998 in view of Suzuki et al, 1972 and Kramer et al, 2001.

In support of their request that the prior rejection of Claims 4, 5 and 10 under 35 U.S.C. § 103(a) as unpatentable over Stumpe in view of Suzuki and Sugimura (1988a) [Kramer et al, 2001]* be withdrawn, Applicants provide the following argument, which is relevant to the rejection above.

(A) In the claimed process, two or three consecutive basic residues are situated from a P10 position to a P3 position of the cleaved polypeptide. In both Stumpe and Suzuki, however, the major disclosed protamine substrates do not have a sequence that would read on this claim element, regardless of where the peptide was cleaved. Any sequence encompassing eight (Stump) or six (Suzuki) basic residues (P10 - P3) would include more than the recited two or three consecutive basic amino acids.

*[Although the first sentence of said rejection cites Sugimura (1988a), the body of the rejection discusses Kramer et al, 2001 as providing evidence that OmpT protease AAA24430.1 can cleave between dibasic residues. Kramer et al was discussed and provided with the prior action. The skilled artisan would have understood that the prior rejection of Claims 4, 5 and 10 under 35 U.S.C. § 103(a) as unpatentable was meant to be over Stumpe in view of Suzuki and Kramer et al.]

(A) Reply: This argument is not found to be persuasive for the following reason. The rejection is not based on cleavage of the protamine disclosed by Stumpe. It is acknowledged that the protamine of Suzuki has six basic residues within P10-P3. Nonetheless, the protamine of Suzuki is encompassed by Claims 36 and 43-46 because said claims do not recite a limitation for the upper number of basic residues within P10-P3.

Claims 36-40 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Stumpe et al, 1998, Suzuki et al, 1972, and Kramer et al, 2001 in view of

Yamamoto et al, 1996 and Dekker et al, 2001. As described above, the combination of Stumpe et al, Suzuki et al, and Kramer et al teaches using OmpT protease AAA24430.1 to cleave the protamine component Clupein YII between ^{23}Arg-Arg-Arg-Ala-Arg↓Arg^{28}, wherein positions P5-P1 are as set forth by SEQ ID NO: 11. Said combination does not teach cleaving a fusion protein comprising the target protein motilin (FVPIFTYGELQRMQEKERNKGQ). Yamamoto et al teaches making a fusion protein comprising the target protein motilin and cleaving said fusion protein using OmpT protease (Col 4-Formula 1a (A-B-C); Col 6, parg5; Col 7, parg2; Example 1). Yamamoto et al does not teach cleaving a fusion protein wherein the C-terminus of a protecting peptide is the P1 position and the N-terminus of motilin is the P1' position. Dekker et al teaches that OmpT protease cleaves at the motif Arg↓Phe-Val (Table 2). It would have been obvious to a person of ordinary skill in the art to combine the teachings of the combination of Stumpe et al, Suzuki et al, and Kramer et al with the teachings of Yamamoto et al and Dekker et al. Said combination would provide a method for cleaving a fusion protein comprising the target protein motilin (FVPIFTYGELQRMQEKERNKGQ), wherein the C-terminus of the protecting peptide is the P1 position and the N-terminus of motilin is the P1' position. In said method, the protecting protein would comprise residues 23-27 of Clupein YII, ^{23}Arg-Arg-Arg-Ala-Arg^{27} (SEQ ID NO: 11) linked directly to the N-terminal P1' Phe^{1} of motilin and OmpT protease AAA24430.1 would cleave between Arg^{27} P1 of Clupein YII and P1' Phe^{1} of motilin: …RRRAR↓F-VPIFTYGELQRMQEKERNKGQ. Motivation to do so is provide by the desire to isolate motilin without additional amino acids. The expectation of success is high, as Kramer et al teaches that Arg at P1 is preferred (Fig4) and Dekker et al teaches that Phe at P1' is allowed and Val at P2' is preferred (Table 2). Therefore, Claims 36-40 are rejected under 35 U.S.C.

103(a) as being unpatentable over the combination of Stumpe et al, 1998, Suzuki et al, 1972, and Kramer et al, 2001 in view of Yamamoto et al, 1996 and Dekker et al, 2001.

> In support of their request that the prior, analogous rejection of Claims 2 and 31-33 under 35 U.S.C. § 103(a) as unpatentable over Yamamoto in view of Dekker be withdrawn, Applicants provide the following argument, which is relevant to the rejection above. Claims 36-40 recite cleaving a polypeptide with an E. coli OmpT protease, wherein two or three consecutive basic residues are situated in the amino acid sequence from a P10 position to a P3 position of the polypeptide. The combination of Yamamoto and Dekker, however, are silent regarding at least this claim element.
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> This argument is not found to be persuasive because the rejection above uses teachings of Suzuki et al to remedy the deficiency in Yamamoto and Dekker, i.e., two or three consecutive basic residues within the P10 to P3 positions.

Claim 41 is rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Stumpe et al, 1998, Suzuki et al, 1972, Kramer et al, 2001, Yamamoto et al, 1996, and Dekker et al, 2001, in view of Yabuta et al, 1995. As described above, the combination of Stumpe et al, Suzuki et al, Kramer et al, Yamamoto et al, and Dekker et al teaches a method for cleaving the fusion protein …RRRAR↓FVPIFTYGELQRMQEKERNKGQ. Said combination does not teach coexpressing the fusion protein and the OmpT protease in the same cell as a means to cleave the fusion protein. Yabuta et al teaches recombinantly expressing a protein, comprising an OmpT cleavage motif, in a cell co-expressing OmpT protease as a means to cleave the protein (Fig4). It would have been obvious to a person of ordinary skill in the art to use the method of Yabuta et al to co-express the fusion protein rendered obvious by the combination of Stumpe et al, Suzuki et al, Kramer et al, Yamamoto et al, and Dekker et al with an endogenous OmpT protease, or an exogenous AAA24430.1 protein, as a means to cleave the fusion protein. Motivation to do so is provide by the desire to cleave said fusion protein without the effort of purifying OmpT protease. The expectation of success is high, as all methods were known in the art and the skilled artisan would believe, based on Yabuta et al, that the fusion protein would be cleaved by the co-expressed endogenous or exogenous OmpT protease. Therefore, Claim 41 is rejected under 35

U.S.C. 103(a) as being unpatentable over the combination of Stumpe et al, 1998, Suzuki et al, 1972, Kramer et al, 2001, Yamamoto et al, 1996, and Dekker et al, 2001, in view of Yabuta et al, 1995.

> In support of their request that the prior rejection of Claims 2, 31, 33, and 35 under 35 U.S.C. § 103(a) as unpatentable over Sugimura (1988a), Okuno (2002a), Okuno (2002b), and Dekker in view of Yabuta be withdrawn, Applicants provide the following argument, which is relevant to the rejection above. None of the references teaches or suggests cleaving a polypeptide with an E. coli OmpT protease, wherein two or three consecutive basic residues are within P10 to P3 of the polypeptide.
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> This argument is not found to be persuasive because the rejection above uses Suzuki et al to teach two or three consecutive basic residues within P10-P3.

Claims 42 and 66 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Stumpe et al, 1998, Suzuki et al, 1972, and Kramer et al, 2001 in view of Dekker et al, 2001 and Okuno et al, 2002b. As described above the combination of Stumpe et al, Suzuki et al, and Kramer et al teaches using OmpT protease AAA24430.1 to cleave the protamine component Clupein YII between ^{23}Arg-Arg-Arg-Ala-Arg↓Arg^{28}, wherein positions P5-P1 are as set forth by SEQ ID NO: 11. Said combination does not teach converting an undesirable OmpT cleavage motif to a non-cleaved motif by setting an acidic amino acid at the P3 position of the undesirable OmpT cleavage motif. Dekker et al teaches that the acidic amino acid Asp or Glu at the P2 position inhibits cleavage by OmpT protease (Table 2). Okuno et al, 2002b teaches that the acidic amino acid Asp or Glu at the P4 position inhibits cleavage by OmpT protease (Table 2). It would have been obvious to a person of ordinary skill in the art to convert any undesirable OmpT cleavage motifs, in the protamine component Clupein YII of Suzuki et al to non-cleaved motifs by setting Asp or Glu at the P3 position of the undesirable OmpT cleavage motif. Motivation to do so is provide by the desire to avoid cleavage at the undesirable motifs, thus producing specific fragments of Clupein YII to be used, for example, in making an antibody. The

expectation of success is high, as based on Okuno et al, 2002b and Dekker et al, the skilled artisan would have believed that, more likely than not, an acidic amino acid at the P3 position would inhibit cleavage by OmpT protease AAA24430.1. Therefore, Claims 42 and 66 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Stumpe et al, 1998, Suzuki et al, 1972, and Kramer et al, 2001 in view of Dekker et al, 2001 and Okuno et al, 2002b.

> Regarding the prior, analogous rejection of Claims 3, 8 and 9 under 35 U.S.C. § 103(a) as unpatentable over Okuno (2002a), Okuno (2002b), or Sugimura (1988a) in view of Dekker and Okuno (2002b), Applicants provide the following argument, which is relevant to the rejection above. Sugimura (1988a) is relied upon for a disclosure relevant to an acidic amino acid at position P3, which is recited in dependent claim 42. None of the references teaches or suggests at least cleaving a polypeptide with an E. coli OmpT protease, wherein two or three consecutive basic residues are situated in the amino acid sequence from a P 10 position to a P3 position, as recited in the claims.
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> This argument is not found to be persuasive because the rejection above uses Suzuki et al to teach two or three consecutive basic residues within P10-P3.

Asp^{97} variants of E. coli proteins having an activity of AAA24430.1

Claim 48 is rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Stumpe et al, 1998, Suzuki et al, 1972, Kramer et al, 2001 in view of Metzler, 2001. As described above, the combination of Stumpe et al, Suzuki et al, and Kramer et al teaches use of OmpT protease AAA24430.1 to cleave the specific protamine component Clupein YII ^{23}Arg-Arg-Arg-Ala-Arg↓Arg^{28}. Said combination does not teach using an OmpT protease AAA24430.1 variant having a substitution at position Asp^{97}. The art teaches that amino acids can be classified based on charge, hydrophobicity, and size (Metzler, 2001). Thus, Asp and Glu are considered to both be acidic amino acids. Kramer et al teaches that Asp^{97} aids in substrate binding by coordinating with the P1' Arg of known OmpT protease -Arg↓Arg- substrates (Fig 4). It would have been obvious to the skilled artisan to make the Glu^{97} OmpT protease variant (conservative substitutions for Asp) and use the variant to cleave the specific protamine

component Clupein YII ^{23}Arg-Arg-Arg-Ala-Arg↓Arg^{28}. Motivation to do so is provide by the desire to demonstrate that the OmpT protease variant cleaves the specific protamine component Clupein YII and to generate fragments of Clupein YII to be used, for example, in making an antibody. The expectation of success is high, as the skilled artisan would have believed that, more likely than not, the Glu^{97} OmpT AAA24430.1 protease variant has the same substrate specificity as the parent Asp^{97} OmpT AAA24430.1 protease. Therefore, Claim 48 is rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Stumpe et al, 1998, Suzuki et al, 1972 and Kramer et al, 2001 in view of Metzler, 2001.

> Regarding the prior rejection of Claim 34 under 35 U.S.C. § 103(a) as unpatentable over Sugimura (1988a), Okuno (2002a), Okuno (2002b), or Dekker in view of Grodberg, relevant to the rejection of Claim 48 above, Applicants provide the same argument discussed above for other rejections i.e., that none of said references teaches or suggests cleaving a polypeptide with two or three consecutive basic residues within the P10-P3 positions. This argument is not found to be persuasive because, as explained above, Suzuki et al teaches two or three consecutive basic residues within P10-P3.

Claim 49 is rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, Dekker et al, 2001, and Kramer et al, 2001 in view of Metzler, 2001. Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al each teach cleavage of a series of polypeptides by OmpT protease. Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al do not teach cleaving said proteins with an OmpT protease AAA24430.1 variant having a substitution at position Asp^{97}. Kramer et al teaches an OmpT protease variant having an Asp^{97}Ala substitution and that said variant cleaves at Ala-Arg↓Arg-Ala (Fig2). It would have been obvious to a person of ordinary skill in the art to determine which of the proteins of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al are cleaved by the OmpT protease Asp^{97}Ala variant of Kramer et al. Motivation to do so is provide by the desire to examine the cleavage motif

requirement of said variant by using the sequences of Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al. The expectation of success is high, as the skilled artisan would have believed that, more likely than not, the OmpT protease $Asp^{97}Ala$ variant of Kramer et al would cleave one or more of the numerous substrates taught by Sugimura et al, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al, including those having a P1' that is, or is not, Arg or Lys. Therefore, Claim 49 is rejected under 35 U.S.C. 103(a) as being unpatentable over Sugimura et al, 1988a, Okuno et al, 2002a, Okuno et al, 2002b, and Dekker et al, 2001 in view of Kramer et al, 2001.

Regarding the prior, analogous rejection of Claims 12-14, and 27-30 under 35 U.S.C. § 103(a), as unpatentable over Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, and Kramer in view of Metzler, Applicants provide the following arguments, which are relevant to the rejection above.

(A) The relevant disclosures of Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, Kramer, and Metzler are set forth above. The combination of references does not teach a process of cleaving a polypeptide with an OmpT protease 97th amino acid variant, where the P1' position is an amino acid other than arginine or lysine.

(A) Reply: It is Sugimura et al and Dekker et al who disclose substrates where the P1' position is an amino acid other than arginine or lysine. Motivation to use the D97A variant of Kramer et al to cleave said substrates derives from the desire to determine the cleavage motif requirement of the variants. Substrates taught by Sugimura et al and Dekker et al, where the P1' position is an amino acid other than arginine or lysine, would be of particular interest since the D97A variant has low efficiency in cleaving the substrate Ala-Arg↓Arg-Ala.

(B) Furthermore, Kramer merely speculates that P1' may cooperate with Asp97 of the E. coli OmpT protease. Kramer states: "Assuming that the substrate has an extended conformation and that the P1 motif chain points toward Glu27 and Asp208, the P1' chain would be located close to Asp97." (pg 429, left col, lines 2-8). There can be no reasonable expectation that replacement of the 97th aspartic acid with other amino acid would have changed substrate specificity, let alone the presently claimed amino acid substitution of Asp97.

(B) Reply: It is acknowledged that Kramer makes said statement. However, Applicants take said statement out of context with Kramer's conclusion, which is: "D97A OmpT displayed only 6% residual activity, therefore we propose that Asp97 is responsible for the observed P1' specificity. A schematic model of a peptide in the active motif is shown in Fig. 4."

Claims 51-56, 59-61, 67, and 68 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Stumpe et al, 1998, Suzuki et al, 1972, Kramer et al, 2001, Yamamoto et al, 1996, and Dekker et al, 2001 in view of Metzler, 2001. As described above, the

combination of Stumpe et al, Suzuki et al, Kramer et al, Yamamoto et al, and Dekker et al teaches a method for cleaving the fusion protein ...RRRAR↓FVPIFTYGELQRMQEKER-NKGQ, wherein the C-terminal Arg of the protecting peptide is in the P1 position and the N-terminal Phe^{1} of motilin is the P1' position, wherein the method uses the OmpT AAA24430.1. Said combination does not teach using an OmpT protease variant having a substitution at position Asp^{97}. Based on the teachings of Metzler, 2001 and Kramer et al, described above, it would have been obvious to the skilled artisan to use the Glu^{97} OmpT protease variant to cleave the fusion protein ...RRRAR↓FVPIFTYGELQRMQE-KERNKGQ. The expectation of success is high, as the skilled artisan would have believed that, more likely than not, the Glu^{97} OmpT protease has the same substrate specificity as the parent Asp^{97} OmpT protease AAA24430.1. Therefore, Claims 51-56, 59-61, 67, and 68 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Stumpe et al, 1998, Suzuki et al, 1972, Kramer et al, 2001, Yamamoto et al, 1996, and Dekker et al, 2001 in view of Metzler, 2001.

None of Applicants' arguments are specifically relevant to the above rejection.

Claims 57 and 58 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Stumpe et al, 1998, Suzuki et al, 1972, Kramer et al, 2001, Yamamoto et al, 1996, Dekker et al, 2001, and Metzler, 2001 in view of Dekker et al, 2001 and Okuno et al, 2002b. As described above, the combination of Stumpe et al, Suzuki et al, Kramer et al, Yamamoto et al, Dekker et al, and Metzler teaches a method using the Glu^{97} OmpT AAA24430.1 protease variant for cleaving the fusion protein ...RRRAR↓FVPIFTYGELQRMQEKERNKGQ, wherein the C-terminal Arg of the protecting peptide is in the P1 position and the N-terminal Phe^{1} of motilin is the P1' position. Said

combination does not teach converting an undesirable OmpT cleavage motif to a non-cleaved motif by setting an acidic amino acid at the P3 position of the undesirable OmpT cleavage motif. Dekker et al teaches that the acidic amino acid Asp or Glu at the P2 position inhibits cleavage by OmpT protease (Table 2). Okuno et al, 2002b teaches that the acidic amino acid Asp or Glu at the P4 position inhibits cleavage by OmpT protease (Table 2). It would have been obvious to a person of ordinary skill in the art to convert any undesirable OmpT cleavage motifs, in the …RRRARFVPIFTYGELQRMQEKERNKGQ fusion protein, to non-cleaved motifs by setting Asp or Glu at the P3 position of the undesirable OmpT cleavage motif. Motivation to do so is provide by the desire to avoid cleavage at the undesirable motifs, thus producing the full-length motilin to be used, for example, in making an antibody. The expectation of success is high, as based on Okuno et al, 2002b and Dekker et al, the skilled artisan would have believed that, more likely than not, an acidic amino acid at the P3 position would inhibit cleavage by OmpT AAA24430.1 protease and the Glu^{97} variant thereof. Therefore, Claims 57 and 58 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Stumpe et al, 1998, Suzuki et al, 1972, Kramer et al, 2001, Yamamoto et al, 1996, and Dekker et al, 2001, and Metzler, 2001 in view of Dekker et al, 2001 and Okuno et al, 2002b.

> Regarding the prior rejection of Claims 18, 23, and 24 under 35 U.S.C. § 103(a) as allegedly unpatentable over Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, Kramer, and Metzler in view of Dekker and Okuno (2002b), relevant to the rejection of Claims 57 and 58 above, Applicants provide the same argument discussed above for other rejections i.e., that none of said references teaches or suggests cleaving a polypeptide with two or three consecutive basic residues within the P10-P3 positions. This argument is not found to be persuasive because, as explained above, Suzuki et al teaches two or three consecutive basic residues within P10-P3.

Claims 49, 50, 54-56, and 63-65 are rejected under 35 U.S.C. 103(a) as being unpatentable over Okuno et al, 2002a and Dekker et al, 2001 in view of Kramer et al, 2001. Okuno et al teaches cleavage by OmpT protease of a series of fusion proteins wherein the P1'

position is not Arg or Lys and the P4 position is a basic residue (Fig1-3; Table 1-3). Dekker et al teaches cleavage by OmpT protease of a series of peptides wherein the P1' position is not Arg or Lys (Table 2). Neither Okuno et al nor Dekker et al teach cleaving said protein sequences with an OmpT protease AAA24430.1 variant having a substitution at position Asp^{97}. Kramer et al teaches an OmpT protease variant having an $Asp^{97}Ala$ substitution and that said variant cleaves, to some extent, at Ala-Arg↓Arg-Ala (Fig2). It would have been obvious to a person of ordinary skill in the art to determine which of the substrates of Okuno et al and Dekker et al are cleaved by the OmpT protease $Asp^{97}Ala$ variant of Kramer et al. Motivation to do so is provide by the desire to examine the cleavage motif requirement of said variant by using the sequences of Okuno et al and Dekker et al. The expectation of success is high, as the skilled artisan would have believed that, more likely than not, the OmpT protease $Asp^{97}Ala$ variant of Kramer et al would cleave one or more of the numerous substrates taught by Sugimura et al and Dekker et al, including those having a P1' that is not Arg or Lys and having a single Arg at either P10-P3. Therefore, Claims 49, 50, 54-56, and 63-65 are rejected under 35 U.S.C. 103(a) as being unpatentable over Okuno et al, 2002a and Dekker et al, 2001 in view of Kramer et al, 2001.

Regarding the prior, analogous rejection of Claims 12-14, 21, and 22 under 35 U.S.C. § 103(a) as unpatentable over Sugimura (1988a), Okuno (2002a), Okuno (2002b), and Dekker in view of Kramer. Applicants make the following arguments, which are relevant to the rejection above. The reasons these arguments are not found to be persuasive are explained in each reply.

(A) Kramer discloses cleavage of a substrate by an OmpT D97A variant (see FIG. 2). Kramer uses the substrate Abz-Ala-Arg↓Arg-Ala-Dap(dnp)-Gly. Claim 49, however, is directed in part to a process for cleaving a polypeptide with an E. coli OmpT protease 97th amino acid variant, where the P1' position is an amino acid other than arginine or lysine. Kramer does not disclose a P1' position amino acid other than arginine or lysine.

(A) Reply: It is Sugimura et al and Dekker et al who disclose substrates where the P1' position is an amino acid other than arginine or lysine.

(B) Nor does Kramer suggest using an OmpT D97A variant to cleave alternate substrates. In fact, the D97A variant cleaved the Abz-Ala-Arg-Arg-Ala-Dap(dnp)-Gly substrate with only 6% efficiency,

relative to wild-type OmpT. This relative inefficiency would not have prompted the skilled artisan to make other OmpT 97th amino acid variants, let alone test such variants with alternate substrates.

(B) Reply: In fact, the low efficiency of the D97A variant in cleaving the OmpT substrate Ala-Arg↓Arg-Ala would motivate the skilled artisan to determine which substrates said variant cleaves with efficiency. It would be obvious to try the substrates taught by Sugimura et al and Dekker et al, including substrates where the P1' position is an amino acid other than arginine or lysine. The skilled artisan would predict that, more likely than not, one or more of said substrates would be cleaved by the D97A variant.

(C) The disclosures of Sugimura (1988a), Okuno (2002a), Okuno (2002b), and Dekker are set forth above. Some of the OmpT substrates disclosed in these references have an amino acid other than arginine or lysine at the P1' position. The Office has not explained, however, why the skilled artisan would have used an OmpT D97A variant showing only 6% of the wild-type activity to cleave any of the substrates disclosed in Sugimura (1988a), Okuno (2002a), Okuno (2002b), and Dekker. In particular, the Office has not explained what would have motivated the artisan to select from all the disclosed substrates only those with a P1' position that is an amino acid other than arginine or lysine, as recited.

(C) Reply: As explained above, motivation derives from the desire to determine the cleavage motif requirement of the D97A variant. Substrates taught by Sugimura et al and Dekker et al, where the P1' position is an amino acid other than arginine or lysine, would be of particular interest since the D97A variant has low efficiency in cleaving the substrate Ala-Arg↓Arg-Ala.

Claims 49-51, 63, and 64 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Okuno et al, 2002a, Dekker et al, 2001, and Kramer et al, 2001 in view of Metzler, 2001. As described above, the combination of Okuno et al, 2002a, Dekker et al, and Kramer et al renders obvious cleavage of one or more substrates, wherein the P1' position is not Arg or Lys, with the Asp^{97}Ala OmpT protease variant of Kramer et al. Said combination does not teach cleavage of one or more of said substrates with an OmpT protease variant having a substitution at Asp^{97} with a residue other than Ala. The art teaches that amino acids can be classified based on charge, hydrophobicity, and size (Metzler, 2001). Thus, (i) Ala, Leu, Phe, and Met are considered to be neutral/hydrophobic amino acids and (ii) Ser, Thr, Cys, Asn, and Gln are considered to be small hydrophobic/polar amino acids. It would have been obvious to a person of ordinary skill in the art to make OmpT protease AAA24430.1 variants having a substitution at Asp^{97} with any of (i) Ala, Leu, Phe, and Met or (ii) Ser, Thr, Cys, Asn, and Gln

and use said variants to cleave one or more substrates of Okuno et al, 2002a and Dekker et al, including those having a P1' that is not Arg or Lys and a single basic amino acid within P10-P3. Motivation to do so is provided by the desire to examine the cleavage motif requirement of said variants by using the sequences of Okuno et al, and Dekker et al. The expectation of success is high, as the skilled artisan would have believed that, more likely than not (i) the OmpT protease variants having a substitution at Asp^{97} with any of Ala, Leu, Phe, and Met would cleave a substrate comprising a P1' of I, F, V, A, Y, M, W, or L and (ii) the OmpT protease variants having a substitution at Asp^{97} with any of Ser, Thr, Cys, Asn, and Gln would cleave a substrate comprising a P1' of S, C, N, Q, T, or G. This expectation of success is based on the fact that (i) neutral/hydrophobic amino acids are more likely to associate with each other, (ii) small hydrophobic/polar amino acids are more likely to associate with each other, and (iii) substrate binding and cleavage is also coordinated by residues Glu^{27}, Asp^{83}, Asp^{85}, Asp^{208}, Asp^{210}, and His^{212} of OmpT protease (Kramer et al; Fig 4). Therefore, Claims 49, 50, 63, and 64 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Okuno et al, 2002a, Dekker et al, 2001, and Kramer et al, 2001 in view of Metzler, 2001.

Regarding the prior rejection of Claims 12-14, and 27-30 under 35 U.S.C. § 103(a), as unpatentable over Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, and Kramer in view of Metzler, Applicants provide the following arguments, which are relevant to the rejection above.

(A) The relevant disclosures of Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, Kramer, and Metzler are set forth above. The combination of references does not teach a process of cleaving a polypeptide with an OmpT protease 97th amino acid variant, where the P1' position is an amino acid other than arginine or lysine.

(A) Reply: As explained above, it is Sugimura et al and Dekker et al who disclose substrates where the P1' position is an amino acid other than arginine or lysine. As also explained above, motivation to use the D97A variant of Kramer et al to cleave said substrates derives from the desire to determine the cleavage motif requirement of the variants. Substrates taught by Sugimura et al and Dekker et al, where the P1' position is an amino acid other than arginine or lysine, would be of particular interest since the D97A variant has low efficiency in cleaving the substrate Ala-Arg↓Arg-Ala. It would also be expected that the D97E OmpT AAA24430.1 variant would have the same specificity as D97 OmpT AAA24430.1.

(B) Furthermore, Kramer merely speculates that P1' may cooperate with Asp97 of the E. coli OmpT protease. Kramer states: "Assuming that the substrate has an extended conformation and that the P1 motif chain points toward Glu27 and Asp208, the P1' chain would be located close to Asp97." (pg 429, left col, lines 2-8). There can be no reasonable expectation that replacement of the 97th aspartic acid with other amino acid would have changed substrate specificity, let alone the presently claimed amino acid substitution of Asp97.

(B) Reply: It is acknowledged that Kramer makes said statement. However, Applicants take said statement out of context with Kramer's conclusion, which is: "D97A OmpT displayed only 6% residual activity, therefore we propose that Asp97 is responsible for the observed P1' specificity. A schematic model of a peptide in the active motif is shown in Fig. 4."

Claims 49, 50, and 65 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Okuno et al, 2002a, Dekker et al, 2001, and Kramer et al, 2001 in view of Metzler, 2001. As described above, the combination of Okuno et al, 2002a, Dekker et al, and Kramer et al renders obvious using the $Asp^{97}Ala$ OmpT protease variant of Kramer et al for cleaving one or more substrates of Okuno et al and Dekker et al, wherein the P1' position is not Arg or Lys. Said combination does not teach cleavage of one or more substrates with the His^{97} OmpT protease variant. The art teaches that amino acids can be classified based on charge, hydrophobicity, and size (Metzler, 2001). Thus, Asp and Glu are considered to both be acidic amino acids, while Arg, Lys, and His are considered to be basic amino acids. Kramer et al teaches that Asp^{97} aids in substrate binding by coordinating with the P1' Arg of the known OmpT protease -Arg↓Arg- substrates (Fig 4). It would have been obvious to the skilled artisan to make the His^{97} OmpT protease variant and use the variant to cleave one or more OmpT protease substrates of Sugimura et al and Dekker et al having a substitution of the P1' position with an acidic amino acid such as Glu and Asp (Okuno et al, Fig 2-3 & Table 2; Dekker et al, Table 2). The expectation of success is high, as the skilled artisan would have believed that, more likely than not, the His^{97} OmpT protease variant would have substrate specificity for a P1' acidic amino acid. This expectation of success is based on the fact that (i) the Asp^{97} OmpT

protease does not cleave substrates having an acidic P1' residue (Okuno et al, Fig 2-3 & Table 2; Dekker et al, Table 2), (ii) the His^{97} basic amino acid of the OmpT protease variant is likely to bind with a P1' acidic amino acid, and (iii) substrate binding and cleavage is also coordinated by residues Glu^{27}, Asp^{83}, Asp^{85}, Asp^{208}, Asp^{210}, and His^{212} of OmpT protease (Kramer et al; Fig 4). Therefore, Claims 49, 50, and 65 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Okuno et al, 2002a, Dekker et al, 2001, and Kramer et al, 2001 in view of Metzler, 2001.

Regarding the prior rejection of Claims 12-14, 21, 22, 27, and 30 under 35 U.S.C. § 103(a), as unpatentable over Sugimura, Okuno (2002a), Okuno (2002b), Dekker, and Kramer in view of Metzler, Applicants provide the following argument, which is relevant to the rejection above. The combination of references does not teach a process of cleaving a polypeptide with an E. coli OmpT protease 97th amino acid variant, where the P1 position is arginine or lysine and the P1' position is an amino acid other than arginine or lysine.

Said argument is not persuasive because, as explained above, the combination of Okuno et al, 2002a, Dekker et al, and Kramer et al renders obvious using the Asp^{97}Ala OmpT protease variant of Kramer et al for cleavage of one or more substrates of Okuno et al and Dekker et al, wherein the P1' position is not Arg or Lys.

Allowable Subject Matter

No claims are allowable.

Applicant's amendment necessitated any new grounds of rejection presented in this Office action. Any new references were cited solely to support rejection(s) based on amendment or rebut Applicants' arguments. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after

the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Regarding filing an Appeal, Applicants are referred to the Official Gazette Notice published July 12, 2005 describing the Pre-Appeal Brief Review Program.

Final Comments

To insure that each document is properly filed in the electronic file wrapper, it is requested that each of amendments to the specification, amendments to the claims, Applicants' remarks, requests for extension of time, and any other distinct papers be submitted on separate pages. It is also requested that the serial number of the application be referenced on every page of the response.

It is also requested that Applicants identify support, within the original application, for any amendments to the claims and specification.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SHERIDAN SWOPE whose telephone number is 571-272-0943. The examiner can normally be reached on 11a-7:30p7 EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Robert Mondesi, can be reached on 571-272-0956. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published application may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on the access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/SHERIDAN SWOPE/
Primary Examiner, Art Unit 1652